AN INTEGRATED MICROFLUIDICS AND MOBILE MICROSCOPY PLATFORM FOR SICKLE CELL DISEASE SCREENING AT THE POINT OF CARE

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ABSTRACT

We have developed a mobile phone microscope and an imaging chip for detecting sickle cell disease at the point of care. We have also optimized the concentration of a chemical oxygen scavenger (sodium metabisulphite) inside the chip to achieve rapid and controllable sickling of RBCs. In addition to identifying individuals with sickle (HbS) hemoglobin, we could distinguish between sickle cell trait and sickle cell disease based on cell shapes.

KEYWORDS: Sickle cell disease, Mobile microscope, Chemical oxygen scavenger, Cell shape

INTRODUCTION

Sickle cell disease is a hereditary condition seen in populations with mutated hemoglobin (HbS). While there is no cure, early diagnosis can prevent childhood mortality and allow better disease management [1]. About 30% of children born with sickle cell disease in India die before reaching adulthood [2]. We need a rapid (<30 min) and affordable diagnostic test, which can be performed in remote endemic regions by health workers with minimal training. We report an integrated microfluidics and mobile microscopy platform to rapidly (within 30 min) diagnose sickle cell disease patients in low-resource settings. We developed an inverted bright-field mobile phone microscope (fig. 1) with 600X total magnification. We also developed a glass and plastic microfluidic chip (fig. 2), instead of using smears, for imaging blood samples at the point of care.

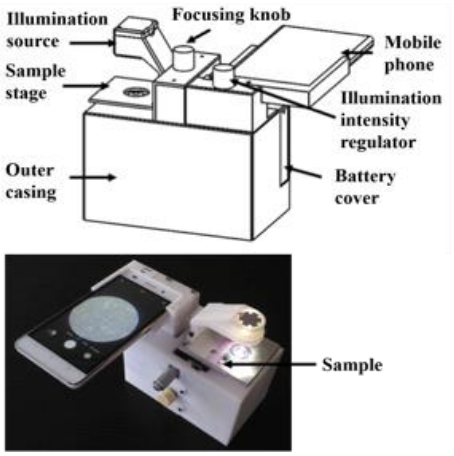

Figure 1: The integrated mobile phone microscope platform. The top image shows a schematic diagram and the bottom image shows a photo.

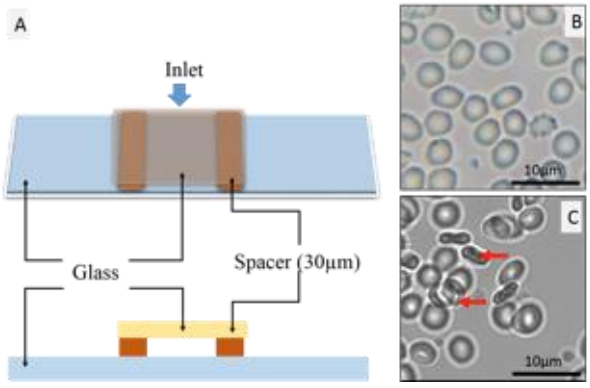

Figure 2: A) Top and side views of the microfluidic chip. B) Image from the chip where RBCs lie correctly oriented (flat side up) in a single layer. C) Image from a smear. Red arrows indicate cells oriented sideways or stacked on top of each other.

EXPERIMENTAL

We have made a glass and plastic microfluidic chip which has a 10mm x 22mm x 30µm imaging chamber for imaging samples. We used a chemical oxygen scavenger, sodium metabisulphite, (SMBS) to generate hypoxic conditions in the blood sample inside a microfluidic chip. Blood taken from a finger prick is diluted 20 times using SMBS solution. The sample is loaded into the chip by capillary flow and the two open ends are sealed with nail lacquer. No RBC staining is performed. We can monitor sickling in real time using the mobile phone microscope. Images of RBCs are taken just

after adding the blood to the chip and again after 30 minutes. These images are analyzed with the open source image processing software ImageJ to extract boundary parameters, such as, area, roundness and solidity of each RBC (fig. 3C). The data for the entire sample is further processed to

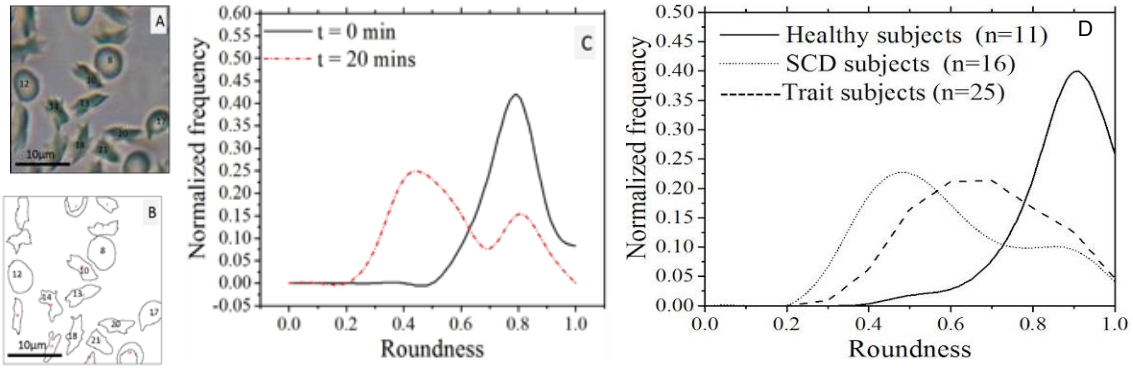

Figure 3: A) Image of unstained RBCs from a sickle cell disease patient taken with the microscope after sickling (~20 min). B) Image for calculating boundary parameters. C) The roundness distribution of the RBCs before (black solid line) and after (red dotted line) sickling. The peak at 0.4 roundness increases after sickling. (D) Characteristic roundness distributions of blood samples from healthy (n=11) individuals, sickle cell disease patients (n =19) and individuals with sickle cell trait (n=21).

obtain a normalized frequency distribution of roundness values (fig. 3D)**.** We have analyzed the blood samples from 52 subjects.

RESULTS AND DISCUSSION

The chemical oxygen scavenger triggers polymerization of HbS and facilitates the shape change of RBCs within 30 min inside the imaging chamber. The height (30μm) of the imaging chamber is crucial to ensure that the RBCs are correctly oriented in a single layer and to allow sickling without any mechanical constraints. RBCs may take up to 24 hours to sickle in traditional blood smears. In contrast, we observe sickling of >80% RBCs in <30 min inside the microfluidic chip. The optical arrangement of the microscope ensures that the final image is projected directly onto the 13MP phone camera (Xiomi Mi3). The microscope can run continuously on battery power for more than a day.

We found that the RBCs from normal (HbA), sickle trait (mixture of HbS and HbA) and sickle cell disease (HbS) individuals have very distinct patterns of roundness (fig. 3D), based on which an unknown blood sample may be classified.

CONCLUSION

We have developed a diagnostic platform that can be deployed in the field for rapid detection of sickle cells, thus eliminating the need for sample transport and avoiding delays in diagnosis. Since the platform uses morphological recognition of sickle cells, there are no false positives similar to solubility tests. We can work with a drop of blood obtained from a finger prick and do not need staining. Finally, we have developed a detection protocol based on experiments with 52 individuals to identify normal, trait and disease subjects within 30 min.

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